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Morphological and physiological plasticity of *Saccharina latissima* (Phaeophyceae) in response to different hydrodynamic conditions and nutrient availability

Guorong Zhu,[1,2,3] Alexander Ebbing,[1] Tjeerd J. Bouma,[1,3*] Klaas R. Timmermans[1*]

[1] Department of Estuarine and Delta Systems, Royal Netherlands Institute for Sea Research (NIOZ), 4401 NT Yerseke, The Netherlands

[2] College of Fisheries, Henan Normal University, Xinxiang, 453007, P.R. China

[3] Faculty of Geosciences, Department of Physical Geography, Utrecht University, 3508 TC Utrecht, The Netherlands

[*] For correspondence. E-mail: tjeerd.bouma@nioz.nl; or Klaas.Timmermans@nioz.nl

Abstract

Morphology and physiology are two key aspects of the adaptation of kelp to varying environments. Some of these kelp responses to co-occurring highly hydrodynamic condition and high nutrient availability are well documented, but little is known about how these factors affect frond surface shape, particularly in the central frond. In this study, morphological and physiological traits of acclimatized *Saccharina latissima* (Phaeophyceae) (three size classes: 44.14±1.15 cm, 29.60±0.75 cm and 16.07±0.45 cm) were compared after 56 days under fully controlled conditions of waves or no waves, and high or low nutrient availability (i.e., LN-NW, LN-W, HN-NW and HN-W treatments). Waves primarily increased frond biomass, elongation rate and carbon to nitrogen ratio (C:N ratio), and induced both a greater variety in and rougher frond surface shapes. The fastest, second-fastest and slowest growth rates were observed in the HN-W, LN-W and LN-NW treatments, respectively. The highest C:N ratio was observed in the LN-W treatment. Together, these results seem to suggest that the thready and spring-like shapes found in the central frond (i.e., rougher frond surface) in wave-exposed conditions can at least partly compensate for low nutrient availability by enhancing nutrient and photon acquisition, particularly in low nutrient conditions. Additionally, large individuals showed significantly larger and heavier fronds compared with other size classes, and the meristematic sections of fronds had the most variance in frond surface shapes and highest C:N ratios compared with distal and mid-sections. Together, these results indicate that frond surface shapes in the newly formed central frond of *S. latissima* can be regarded both as possessing high morphological and physiological plasticity that enables kelp to cope with contrasting environments.

Key words frond surface shape, indicator, plasticity, hydrodynamics, nutrient availability, *Saccharina latissima*

Introduction

Kelp are dominant and essential components of coastal ecosystems around the world (Mann 1973; Harley et al. 2012). However, they are being strongly influenced by a multitude of environmental parameters, including high-energy hydrodynamics and eutrophication, which are two major factors affecting the growth, distribution and even survival of kelp (e.g., Kain 1962; Eriksson et al. 2002; Strain et al. 2014; Bekkby et al. 2019). In general, every environmental parameter can act as a resource or a stress, depending on the intensity/concentration in combination with the environmental setting. For example, water motion from currents and/or waves can, besides imposing hydrodynamic forces (Denny 2006), also increase the uptake of nutrients by reducing the diffusive boundary layer around the surface of kelp (Wheeler 1988; Hurd 2000; Hurd and Pilditch 2011). In other words, water motion probably intensifies the effects of eutrophication or its interaction with other stressors (Strain et al 2014) but may be able to compensate for nutrient limitations. With climate change and increased human activities, hydrodynamics and eutrophication are expected to become more intensive and severe (Katavić 2006; IPCC 2013; Buck et al. 2017).

Kelp may use two key responses to cope with varying hydrodynamic exposure and/or nutrient availability: morphological and physiological plasticity (Gagné et al. 1982; Koehl et al. 2008; Boderskov et al. 2016; Vettori and Nikora 2017; Coppin et al. 2020). Kelp generally exhibit narrow, thick and flat fronds (blade, lamina, Kain 1976) when inhabiting rapidly flowing waters but produce wide, thin and undulating fronds at sheltered sites (Gerard 1987; Buck and Buchholz 2005; Koehl et al. 2008; Vettori and Nikora 2017; Visch et al. 2020). Transplanting kelp from sheltered environments to wave-exposed sites and vice versa has been found to induce these morphological responses (Fowler-Walker et al. 2006; Koehl et al. 2008). Studies on the morphological responses of kelp to nutrient availability have mainly focused on growth (e.g., surface area, biomass, frond elongation) rather than other morphological traits (e.g., frond width, shape), but growth responses are complex. For example, in a 49-day experiment during fall and early winter, *S. accharina latissima* grown under high nutrient availability exhibited relatively faster frond elongation, slightly larger biomass, slow biomass growth rate but similar frond area-specific dry weight when compared with those grown under low nutrient availability (Boderskov et al. 2016). These responses can be explained through the consideration that kelp growth (e.g., frond surface areas, biomass or length) depends on the balance between the production of new tissue and the loss of tissue from the distal end of the frond through erosion or breakage (Krumhansl and Scheibling 2011).

In terms of physiology, the carbon to nitrogen ratio (i.e., C:N ratio) in fronds is an important indicator of seaweed quality (Russell-Hunter 1970; Schaal et al. 2009). The C:N ratio has been found to be reduced by nutrient-enrichment (Hepburn et al. 2007; Stephens and Hepburn 2014, 2016), or the rate of kelp productivity and biomass (i.e., favorable growth seasons, Mann 1972; Niell 1976; Jackson 1977; Stephens and Hepburn 2014), but exhibits complex response to rough hydrodynamics. For example, *Macrocystis pyrifera* exposed to waves displayed reduced C:N ratio in canopy blades but increased in subcanopy blades (Hepburn et al. 2007), and reduced C:N ratio in summer but increased in winter (Stephens and Hepburn 2014). However, few studies have addressed the coupled high hydrodynamics-high nutrient interactions on the morphological and physiological plasticity of kelp (e.g., Strain et al. 2014). To date, the long-term effects of hydrodynamics and nutrients remain unclear. Similarly, it is unclear how high hydrodynamic forces compensate for low nutrient conditions besides reducing the thickness of diffusive boundary layer. This lack of knowledge ultimately hampers insight into how kelp adapt to habitats with varying and co-occurring hydrodynamic exposure and nutrient availability.

Previous morphological studies have mainly focused on the frond width (narrow or wide), edge shape (flat or ruffled) and the general frond surface shape (i.e., surface topographic features, Hurd and Pilditch 2011) in the central frond (i.e., bullations or corrugations, Fig. 1). This focus is clear in studies such as Parke (1948), Druehl and Kaneko (1973), and Vettori and Nikora (2017) for *S. latissima*; Koehl and Alberte (1988), and Koehl et al. (2008) for *Nereocystis luetkeana*; Hurd and Pilditch (2011) for *M. pyrifera*; and Klochkova et al. (2017) for *Tauya basicrassa*. However, frond surface shape in the central frond is complex. There are more than one type of shape and differential changes along the frond (our field and experimental observations) when adapting to different environmental conditions. Thus, intensive study of the responses of frond surface shape, especially in the central frond, is important for fully understanding the morphological adaptions of kelp to varying environmental conditions.

With the globally growing demand for kelp as a source for food, feed, or energy (Adams et al. 2009; Handå et al. 2013) and the increase in other established and emerging uses of coastal areas (e.g., fishing and recreational activities, Troell et al. 2009; Jansen et al. 2016), there is increasing awareness of the need to develop open-sea aquaculture of kelp. Based on the conspicuous and rapid morphological and physiological variations of kelp in response to local environmental conditions, this plasticity could be the vital for achieving both high biomass and quality for future kelp farms in the open sea, and plasticity responses might serve as a potential indicator for assessing the suitability of sites for farming.

S. latissima, a foundation seaweed species of temperate coastal areas, is abundant in sheltered, moderately wave-exposed areas and less abundant on rough, wave-exposed shores (Merzouk and

Johnson 2011; Peteiro and Freire 2013). It grows in the relatively higher nutrient period from late spring to early summer (i.e., a winter-spring species, Niell 1976; eutrophication-tolerant species, Conolly and Drew 1985). In this study, we used *S. latissima* as a species of interest to evaluate the plasticity in morphological and physiological traits in response to co-occurring high hydrodynamic exposure and high nutrient availability. In this study, we assessed the morphological and physiological responses in fronds of three size classes of *S. latissima* to varying hydrodynamic exposures and nutrient availability for 56 days under fully controlled conditions. We hypothesize the following: (1) besides the growth, general morphology, and physiology, the frond surface shape of *S. latissima* will also vary with different levels of hydrodynamic exposure and nutrient availability, accompanied by significant interactions on all these parameters, and (2) that this variation, especially in the frond surface shape, will differ along the longitudinal sections of the frond and among the three size classes. That is, we expect that the meristematic section of *S. latissima* will show the highest levels of morphological and physiological plasticity, especially in frond surface shape, as well as in small individuals. We finally hypothesize that (3) there is no "standard" *Saccharina* morphology because waves can compensate for low nutrient concentrations by forming more complex and rougher frond surfaces.

Materials and methods

Saccharina latissima sample collection

S. latissima individuals were obtained from Zeewaar, which was the first seaweed farm established in the Netherlands and is found at the boundary between the North Sea and the Eastern Scheldt

(51º35’53.5”N; 3º41’04.0”E), in March 2017, 10 days before starting the experiment. All the individuals were kept in natural Eastern Scheldt seawater and were transported immediately (within an hour after collection) to the Netherlands Institute for Sea Research laboratory (Yerseke, the Netherlands, 51º29’17.4”N; 4º3’26.0”E), where the experiments were conducted. After arrival, individuals were kept in a tank with filtered, aerated seawater from the Eastern Scheldt, and natural sun light conditions in a greenhouse for an acclimation period before starting the experiment. From the pool of individuals, 96 individuals were randomly selected and divided into three size classes based on their frond length, i.e. large individuals (average 44.14±SE 1.15 cm), medium individuals (29.60±0.75 cm), and small individuals (16.07±0.45 cm, n=32). Each *Saccharina* individual was composed of an intact frond, stipe and holdfast.

Experimental design

Individual kelps of 3 different size classes were exposed over 56 consecutive days to the following environmental conditions: waves (W) versus no waves (NW), in a full factorial combination with high (HN) versus low (LN) nutrient availability (Table 1). This resulted in four experimental treatments: (1) low nutrient-no wave (LN-NW), (2) low nutrient-wave (LN-W), (3) high nutrient-no wave (HN-NW), and (4) high nutrient-wave (HN-W). Two replicate tanks per treatment were installed, resulting in a total of eight tanks. *Saccharina* individuals were taped to eight flexible sticks (length of 85 cm, diameter of 1.60 cm), which were then fixed in the eight tanks (1 stick per tank, Fig. 2). Each stick contained 12 *Saccharina* individuals, with a recurring sequence of large, medium and small individuals.

Four big tanks (length 350 × width 90 × height 80 cm containing approximately 2500 liters) were equipped with a hydraulic wave generator (operated for 24 h daily, La Nafie et al. 2012) for creating waves. To prevent occurrence of standing waves, we set the system to give approximately every 20 s a quick (5 s) push of the wave paddle followed by a slow (15 s) retreat. This resulted in a large wave, followed by a series of attenuating reflecting waves. The resulting chaotic wave pattern mimics the hydrodynamics driving the back and forth flapping of fronds. The maximum wave flow velocity was approximate 0.33 m s^{-1} (Druck PTX 1830 pressure sensor). The other four tanks (length 110 × width 90 × height 60 cm containing approximately 600 liters) did not have a hydraulic wave generator (no wave treatment). In all tanks, the height of the sticks from the bottom was 20 cm and the water height was maintained at approximately 40 cm.

All tanks were filled with filtered (0.2 µm pore size) seawater from the Eastern Scheldt. The ambient nutrient concentration of this seawater was used as the low-nutrient availability treatment (Table 1). For the high-nutrient availability treatments, $NaNO_3$ and K_2HPO_4 were added to this seawater to a final average concentration of 30 µmol L^{-1} nitrate and 5 µmol L^{-1} phosphate twice a week (Table 1, Fig. S1). These concentrations are saturating for N and P uptake kinetics in *S. latissima* (Lubsch and Timmermans 2019). Dissolved nutrient concentrations were measured on a SEAL-QuAAtro autoanalyzer (Seal, Norderstedt, Germany) after filtering through a glass-fiber filter (0.45 um). Nutrient addition levels are based on and similar to concentrations recorded in eutrophic areas (Kristiansen and Paasche 1982). Continuous aeration was supplied to assure complete mixing of the water column in all tanks. Abundance of microalgae in the tanks was kept low or prevented by the presence of living oysters, actively filtering the water.

Every week, fragments of detached seaweeds from each tank were collected by carefully scooping them up with a net. Light irradiance and water temperature were measured during the experiment with a temperature-light logger (30min per record). Other physical properties of the water column (i.e., conductivity, salinity, pH and suspended solids) were measured by using a conductivity meter (CONSORT K912), a standard pH meter (PHM 210 Meter lab pH meter, Radiometer, Denmark), as well as by measuring the dry weight of the residue on the glass-fiber filter (0.47 um) at the end of experiment. During the experimental period, these environmental conditions were sufficient to allow for growth in *S. latissima* (Light irradiance—80.66±3.01 μmol photons $m^{-2}s^{-1}$, n=8440; water temperature—15.08±0.06 ºC, n=14208; conductivity—48.10±0.15 ms cm^{-1}, n=8; salinity—35.18±0.12 PSU, n=8; pH—8.99±0.13, n=8; suspended solids—197±5 mg L^{-1}, n=16; and dissolved oxygen—9.38 ± 0.46 mg L^{-1}, n=8).

***S. latissima* response parameters**

General morphology—At the end of the experiment, all individuals were carefully harvested to keep each frond, stipe and holdfast intact. Using a measuring tape (precision ± 1.00 mm), we then collected morphometric measurements on the following parameters: stipe length, frond length and frond width (the widest point of the frond).

Frond surface shapes—Determining the frond surface shape of the central frond in *S. latissima* individuals was a much more complex process than used in previous studies (i.e., not only the general shape, namely bullations or corrugations in Druehl and Kaneko 1973; Gerard 1987; Koehl and Alberte 1988; Koehl et al. 2008; Hurd and Pilditch 2011; Klochkova et al. 2017; Vettori and Nikora 2017). According to the dimension and pattern of the central frond surface shape in the 96 individuals, a total of seven types of shapes (bubble, thready, smooth, scattered, and spring-, net-, and bowl-like) were distinguished at the end of the experiment (Figs. 1 and 3). The shape occurrence (i.e., the percentage of each shape out of the total numbers of all kinds of shapes in one of three sections along the frond) was used to analyze the responses of frond surface shape to the experimental treatments (Table 2).

Before analysis, the frond of the thallus from stipe to tip was uniformly divided into three test regions (meristematic section, mid-section, and distal end), and categorized according to the length and shape (Table 2).

Growth performance—Growth performance was assessed by measuring frond biomass (dry weight) and frond elongation rate. To determine frond biomass, after measuring the morphological parameters after harvest, the intact frond of each individual was separated, carefully washed with deionized water (to remove the salt attached to fronds), freeze-dried and dry weight was determined using an electronic analytical balance (precision±0.1 mg). For frond elongation rate, according to Mann (1973), *S. latissima* undergoes intercalary growth with maximum growth occurring between the stipe/frond junction and approximately 10 cm up the frond. Thus, we punched 0.2 mm diameter holes in the meristematic region of each frond at 10 cm above the stipe/frond junction by using the hole punch technique (Parke 1948), and used the following equation to calculate frond elongation rate:

Frond elongation rate = $(l - l_0)/(t_2 - t_1)$,

where l is the distance from the punched hole to the stipe/frond junction at the end of the experiment, l_0 is the original distance from the punched hole to the stipe/frond junction when punched, t_2 is the time at harvest, and t_1 is the time when the hole was punched.

Physiological traits — For physiological traits, we measured the C:N ratio in frond tissues. First, the central frond of the thallus from stipe to tip was uniformly divided into three test regions (meristematic section, mid-section, and distal end), as we did for determining frond surface shape (Table 2). Then each section was freeze-dried and ground before the C:N ratio test, which was conducted using an Elemental Analyzer (Flash 1112, Thermo Scientific). Lyophilized and ground samples were combusted at 1020°C under oxic conditions. The nitrous oxides were reduced to N_2 with elementary copper at 650^{o}C and water was removed by trapping. After separation on a Haysep Q column, CO_2 and N_2 were detected with a Thermal Conductivity Detector detector. The C:N ratio was then calculated by dividing the total carbon content by the total nitrogen content.

Statistical analyses

Statistical analyses were performed using the software program IBM SPSS Statistics 13.0. For growth performance and morphological traits, we used two-way analysis of covariance (ANCOVA), with the size class as the covariate, to analyze the differences among the four treatments. For frond surface shape and C:N ratio, two-way ANCOVA was also conducted to test the effects of wave exposure and nutrient availability, with size class and frond section as the covariates. Then, multiple comparisons of means were performed using Duncan's post hoc test to identify differences in growth performance and morphological traits among all the treatments within each size class, and differences in the frond surface shape and C:N ratio for each size class and frond section, respectively. Two-tailed *P*-values are presented throughout and significance was assumed at the 95% confidence limits of the effect estimates. Before performing ANCOVA, all data were tested for normality and homogeneity of variance. Data were transformed [square(x), ln(x), ln(x+1), cube (x), square root (x), or/and reciprocal(x)] to obtain normality and/or homogeneity of variance, if necessary.

Results

Morphology

In general, high nutrient availability and wave exposure significantly increased the size of all three general morphological traits: stipe length, frond length and frond width (Tables 3 and S1, Fig. 4). Wave exposure induced narrow fronds under high-nutrient conditions, while when under low-nutrient conditions resulted in wide ones (Tables 3 and S1, Fig. 4). Larger size classes had significantly higher frond length and frond width in almost all treatments, as well as the stipe length in the LN-NW treatment (Tables 3 and S3a, Fig. 4).

Frond surface shape occurrence

At the beginning of the experiment, the two previously found types of frond surface shapes (bubble and smooth, Fig. 3) were observed in *S. latissima* individuals. Both of these persisted under most treatments, size classes and frond sections, especially in the sections that were already present at start of experiment, i.e., the distal part at the end of the experimental period (Tables 4, S3b and S4; Figs. 3 and 5). In contrast, five other types of surface shapes (thready, spring, net, bowl and scattered; Fig. 3) observed at time of harvest were generally found in the meristematic section, with some types (thready, spring and scattered) also found in the mid-section (Tables 4 and S4, Figs. 3 and 5). There were no significant differences in surface shapes among the three size classes (Table S3b). Wave exposure significantly increased the frequency of thready and spring-shaped fronds, but generally decreased the occurrence of the bubble type, except for in mid-sections under low nutrient conditions (Tables 4 and S2, Figs. 3 and 5). The significant effects of nutrient availability were only found in few types and depended on wave exposure. For example, high nutrient availability induced more thready and net-shaped meristematic sections and spring-shaped mid-sections when exposed to waves. In contrast, high nutrients resulted in lower frequency of thready and net-shaped sections under no waves. No significant differences were observed in the occurrence of other types of frond surface shapes (bowl, smooth and scattered) among the four treatments (Tables 4 and S2, Figs. 3 and

5).

Growth performance

Frond biomass was significantly positively affected by size classes in all treatments, but was only strongly enhanced by waves (Tables 3, S1 and S3a; Fig. 6). Frond elongation rates were not significantly affected by size class, but were strongly and positively affected by waves, high nutrient availability, and their interactions (Tables 3, S1 and S3a; Fig. 6). All three size classes of individuals presented their highest frond biomass and frond elongation rate under the HN-W treatment and the lowest growth under the LN-NW treatment (Table S1, Fig. 6).

Physiological traits

Frond C:N ratio did not vary significantly among the three size classes but significantly decreased from the meristematic section to distal end (Tables 4, S3b and S4; Fig. 7). High nutrient availability significantly decreased the C:N ratio, particularly when combined with waves (Tables 4 and S2, Fig. 7). Wave exposure, however, significantly increased the frond C:N ratio of all frond sections and size classes, particularly under the low nutrient availability condition, with the highest C:N ratio found under the LN-W treatment for all three size categories and frond sections (Tables 4 and S2, Fig. 7).

Discussion

Here, we demonstrate the morphological (i.e., elongation rate, length, biomass, width, frond surface shape, and the stipe length) and physiological (i.e., C:N ratio) plasticity of *S. latissima* under fully controlled conditions of hydrodynamics and nutrient availability. The main results show that there was no “standard” *Saccharina* morphology. Moreover, we found that waves can induce morphological changes that compensate for low nutrient concentrations. That is, the presence of waves induced more complex and roughly-shaped frond surfaces, through which the increased surface area may capture more light and nutrients, as indicated by larger values of frond biomass and frond elongation rate in the LN-W treatment than in LN-NW or HN-NW treatments. Due to its high levels of morphological plasticity, many different frond surface shapes can be induced by wave exposure, particularly in newly forming sections.

Effects on growth

In this study, we found that both wave exposure and high nutrient conditions could, on their own, promote *S. latissima* growth during the 56–day trial. This result agrees with many other studies on kelp response to either hydrodynamics or nutrient availability as a single environmental factor: rough hydrodynamics (e.g., *S. latissima*, Gerard 1987; *M. pyrifera*, Hepburn et al. 2007) or eutrophic conditions (e.g., *Laminaria digitata* and *S. latissima*, Conolly and Drew 1985). Additionally, we observed the highest frond biomass and frond elongation rates under the HN-W treatment, implying that rough hydrodynamic conditions when paired with high nutrient conditions are more advantageous for *S. latissima* growth than one of the factors by itself, i.e., there is a positive interaction or a synergistic effect. This might be the case because *S. latissima* has a relatively large tolerance to high nutrient availability (Conolly and Drew 1985), thus can take advantage of other environmental factors increasing nutrient availability, such as hydrodynamics (Gerard 1982).

Morphological plasticity

Besides the edge shape, frond surface shape is also a crucial morphological characteristic of kelp in response to hydrodynamic conditions (Koehl et al. 2008; Vettori and Nikora 2017). In our study, we observed five types of shapes that had not been described before, namely the thready, scattered, and spring-, net-, and bowl-like shapes (see Table 2; Figs. 3 and 5). These shapes were frequently observed in our experiment in the newly formed sections, in addition to earlier described general and classical shapes like smooth or bubble shapes (e.g., *S. latissima*, Vettori and Nikora 2017; *N. luetkeana*, Koehl and Alberte 1988, Koehl et al. 2008; *M. pyrifera*, Hurd and Pilditch 2011). Two distinct shapes (thready and spring-shaped) were more often observed under wave-exposed conditions, independent of nutrient condition; concurrently, the occurrence of these same shapes, thready and spring-shaped fronds, increased under high nutrient availability only when paired with waves. This means that we can accept hypotheses 1, 2 and 3. To our knowledge, this is the first report on kelp that documents the ability to change the central frond surface shape in response to ambient conditions.

Physiological plasticity

The C:N ratio can be used to indicate the quality of seaweed for food and feed (Russell-Hunter 1970; Schaal et al. 2009), and has been shown to change with varying abiotic environments (e.g., Hepburn et al. 2007; Schaal et al. 2009; Stephens and Hepburn 2014, 2016; Visch et al. 2020). In the present study, waves increased the C:N ratio, especially under low nutrient conditions. This agrees with the results reported by some other studies on kelp (e.g., *L. digitata*, Schaal et al. 2009; subcanopy blades of *M. pyrifera*, Hepburn et al. 2007; *M. pyrifera* in winter, Stephens and Hepburn 2014; *S. latissima*, Visch et al. 2020). This increase in the C:N ratio of *S. latissima* could be due to the low nitrogen concentration in the LN treatment in this study, and in the experimental sites in the study by Visch et al. (2020), which may limit nitrogen storage in kelp tissue (both was below 10 uM external NO_3^-, the limit concentration to form internal nitrogen reserves for *S. latissima*, Chapman et al. 1978). Meanwhile, waves increase the uptake of nutrients (here, inorganic carbon, nitrogen and phosphorus) and carbon dioxide for photosynthesis, by reducing the thickness of the diffusive boundary layer around the surface of kelp (Wheeler 1988; Hurd 2000; Hurd and Pilditch 2011). Overall, this may result in enhanced carbon storage in kelp tissue, reflected in an elevated C:N ratio. In contrast to waves, high nutrient availability decreased the C:N ratio of *S. latissima* individuals, which agrees well with observations in other kelp species (e.g., *M. pyrifera*, Hepburn et al. 2007; Stephens and Hepburn 2014, 2016). Together with the decreasing C:N ratio found along the frond from the meristematic section to distal end, these results suggest that *S. latissima* could display a relatively high nutritional quality as feed (below the critical 17:1 C:N ratio, to meet the nutritional requirement of a consumer to sustain growth, Russell-Hunter 1970) with eutrophication, but-relatively low quality when exposed to waves. Given the relatively low nutrient and fierce waves in open sea compared to the coastal zones, this implies that *S. latissima* cultured in open sea could have high nutritional values for both food and feed.

Implication plasticity

Frond shapes, such as ruffles, wrinkles, and bullations, have been shown to affect a variety of aspects of performance. For example, fronds with ruffles, wrinkles, and bullations can flutter erratically in flowing water (Koehl et al. 2008) and counter more turbulence in the water flowing across them (Hurd and Stevens 1997; Hurd 2000; Roberson and Coyer 2004), thereby reducing self-shading and enhancing uptake of nutrients. The plasticity of general morphological features in response to wave exposure, i.e., formation of flat and narrow fronds (Gerard 1987; Buck and Buchholz 2005; Koehl et al. 2008;-Vettori and Nikora 2017), however, is disadvantageous for harvesting light and nutrient uptake because of the relatively smaller surface area available to come in contact with the ambient environment than in wide fronds with ruffled edges (Koehl and Alberte 1988). In this study, the increased occurrence of thready and spring-like shapes in the central frond found under the wave-exposed condition could increase the actual frond surface area, similarly to ruffles, wrinkles, and bullations, and be advantageous for light harvesting and nutrient uptake. This in turn can compensate for some of the disadvantages caused by the general morphological features and provide a net benefit for growth. In our study, this is supported by the results that the fastest, second-fastest and the slowest growth rates of *S. latissima* were observed in the HN-W, LN-W and LN-NW treatments, respectively. This means that we can accept hypothesis 3 that waves can compensate for low nutrient concentrations by stimulating more complex and rougher-shaped frond surfaces, which form larger surface areas and result in increased light harvesting and nutrient uptake.

Kelp morphology also has critical implications for the likelihood of dislodgment or survival (i.e., hydrodynamic performance) when experiencing intense hydrodynamic forces (Denny 2006). Shapes along the frond edge, such as ruffles formed in sheltered habitats, increase the hydrodynamic drag on fronds, whereas the flat-edged, relatively long and narrow fronds (i.e., streamlined morphology) formed under wave conditions lead to relatively small hydrodynamic drag force (*S. latissima*, Gerard 1987, Buck and Buchholz 2005; *N. luetkeana*, Koehl et al. 2008). However, within species, size has more important positive consequences on hydrodynamic forces than frond shape, especially under high wave exposure (e.g., 4.0 m s^{-1} flow velocity for *Hedophyllum sessile*, Milligan and deWreede 2004; 2.5-3.0 m s^{-1} orbital water velocities for *Ecklonia radiate*, Bettignies et al. 2013). For example, self-pruning is an important strategy to reduce size (Milligan and deWreede 2004; Demes et al. 2013). Both streamlined morphology and size reduction, as adaptions to waves, were observed in our present study, as indicated by the flat frond edge, significantly large increases in frond elongation rate and relatively long fronds found under wave exposure (also note the higher breakage in the

LN-W treatment). Frond width is another parameter determining drag irregardless of flow speed (e.g., 1.0-3.0 m s^{-1} flow for *E. radiata*, Bettignies et al. 2013), but it did not significantly differ between the wave and no wave treatments in our study.

CONCLUSIONS. With the globally increasing demand for seaweeds as sources of food, feed, and energy (Adams et al. 2009; Handå et al. 2013), the intensive use of coastal areas (Troell et al. 2009; Jansen et al. 2016), and the increasing threat to coastal habitats for kelp (Strain et al. 2014; Bekkby et al. 2019), there is increasing awareness of the need to develop open sea seaweed aquaculture and to reform coastal habitats. *S. latissima*, is the most commonly cultivated European brown algae species, and can be cultivated under more exposed hydrodynamic (open sea) areas than its natural habitat, provided that suitable attachment substrate and planting depth are present (Buck and Buchholz 2005; Azevedo et al. 2019). Our experiment provides further support for the feasibility of kelp aquaculture in more exposed environments such as the open sea, as indicated by the high frond biomass, fast frond elongation rates and low C:N ratio of *S. latissima*, as well as high plasticity in frond surface shape, to high hydrodynamic exposure. Give its highly efficient removal of nutrients from water and its multitude of commercial uses, as well as the preferable association of open sea seaweed aquaculture with fish aquaculture farms (Troell et al. 2009; Buck and Langan 2017; Azevedo et al. 2019), *S. latissima* can be used as a biogenic habitat former in coastal ecosystems. We predict that it will be a very advantageous species in commercial monoculture or integrated multi-trophic aquaculture (IMTA) systems in the open sea, able to grow and thrive with high nutritional values under both eutrophic and stormy conditions.

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Conflict of Interest

None declared

Figure captions

Figure 1. Schematic diagram of the morphological shapes found in previous studies and the present study, mainly focused on frond width (narrow or wide), frond edge shape (flat or ruffled) and the most general frond surface shapes in the central frond (named bullations or corrugations in previous studies), as well as the six additional frond surface shape patterns (thready, spring, net, bowl, flat and scattered; lower right panel; see photos in Fig. 3) defined in this study. An intact individual of *Saccharina latissima* is shown in the lower left panel.

Figure 2. Experimental design, consisting of 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HL: high nutrient availability), each one with 2 independent replicates (flume tanks). Each stick contained 12 individuals, with a recurring sequence of large, medium and small individuals. See ‘Materials and methods’ for more details.

Figure 3. Photographs of fronds (A) and frond surface shapes (B) of *S. latissima* under 2 hydrodynamic treatments (No Wave and Wave) and 2 nutrient treatments (LN—low nutrient availability and HN—high nutrient availability). See ‘Table 2’ for more details.

Figure 4. Morphological traits (stipe length, frond length~~,~~ and frond width~~ and stipe length~~) of *S. latissima* under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability). See ‘Materials and methods’ for more details. Bars represent mean values±1SE (n=8). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan’s multiple range test for each size.

Figure 5. The occurrence of frond surface shapes in *S. latissima* under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments at the end of the experiment (LN: low nutrient

availability and HN: high nutrient availability). See ‘Materials and methods’ for more details. Bars represent mean values±1SE (n=4,6,7,8). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan’s multiple range test for each size and each frond section.

Figure 6. Growth (frond biomass and elongation rate) of *S. latissima* under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability). See ‘Materials and methods’ for more details. Bars represent mean values±1SE (n=8). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan’s multiple range test for each size.

Figure 7. The C:N ratio in fronds of *S. latissima* under 2 hydrodynamic treatments (NW: no wave and W: wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability) (See ‘Materials and methods’ for more details). Bars represent mean values±1SE (n=4). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan’s multiple range test for each size and each frond section.

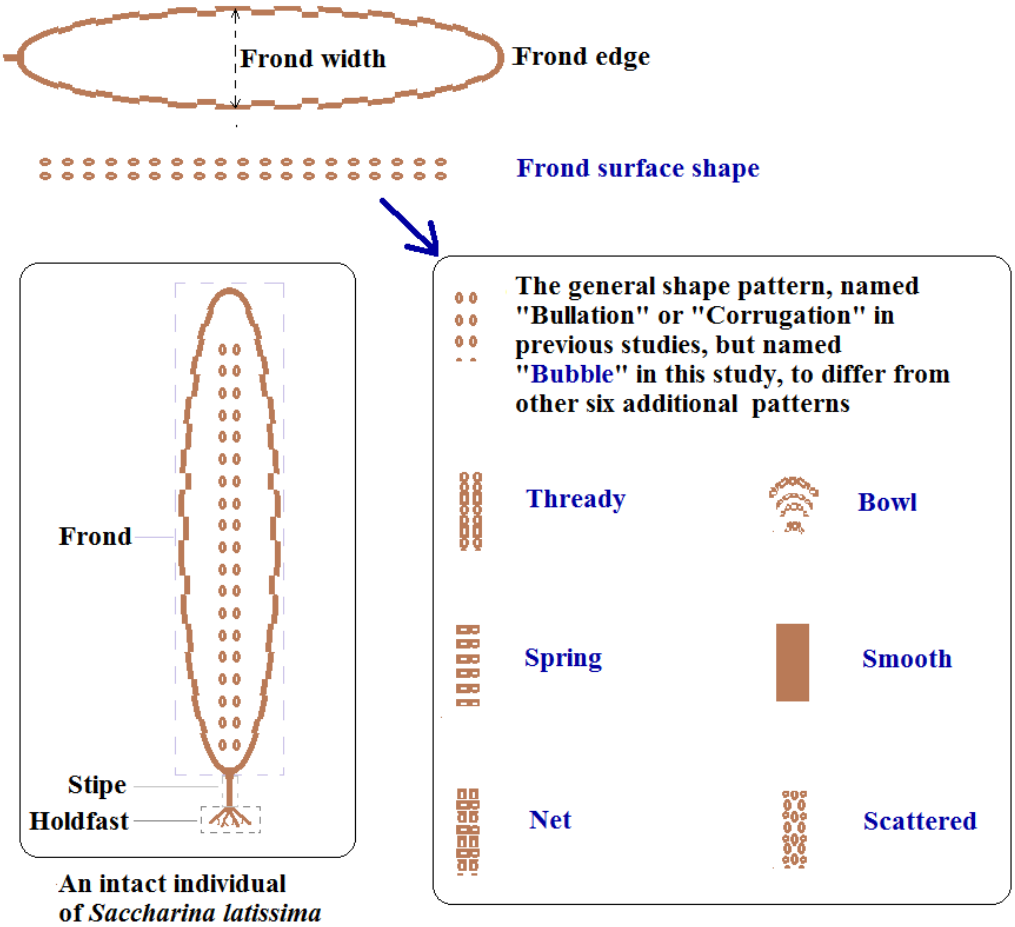

Fig. 1 Schematic diagram of the morphological shapes found in previous studies and the present study, mainly focused on frond width (narrow or wide), frond edge shape (flat or ruffled) and the most general frond surface shapes in the central frond (named bullation or corrugation in previous studies), as well as the six additional frond surface shape patterns (thready, spring, net, bowl, smooth and scattered; lower right panel; see photos in Fig. 3) defined in this study. An intact individual of *Saccharina latissima* is shown in the lower left panel.

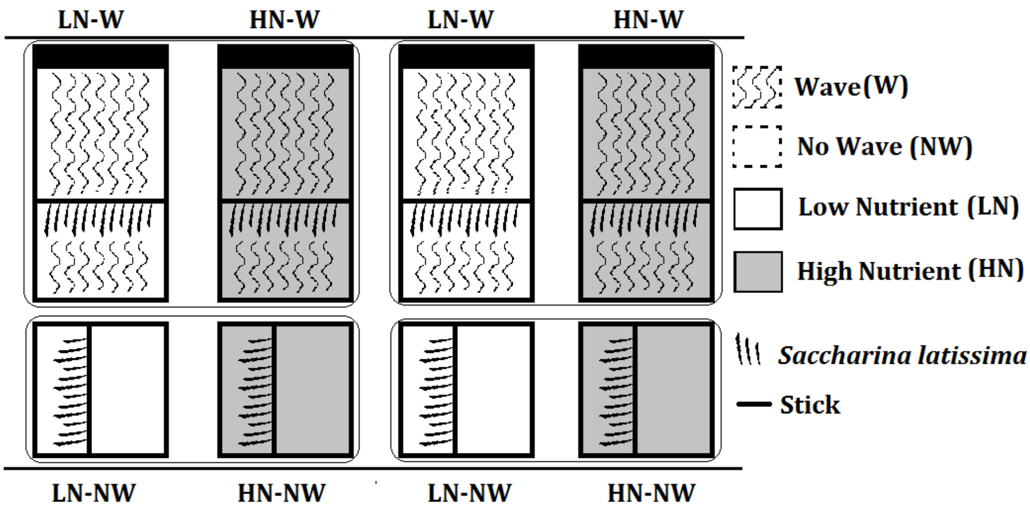

Fig. 2 Experimental design, consisting of 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HL: high nutrient availability), each one with 2 independent replicates (flume tanks). Each stick contained 12 individuals, with a recurring sequence of large, medium and small individuals. See 'Materials and methods' for more details.

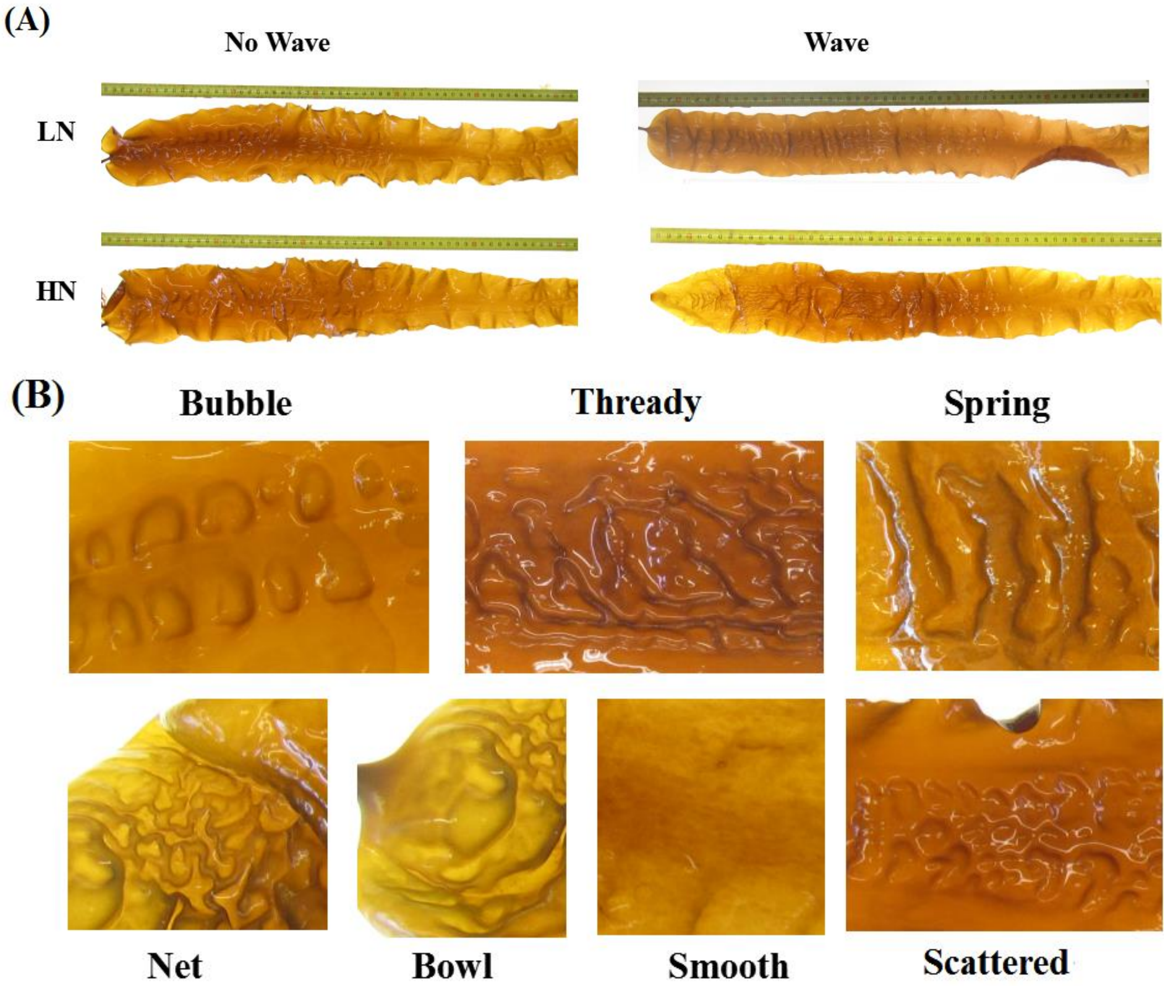

Fig. 3 Photographs of fronds (A) and frond surface shapes (B) of *S. latissima* under 2 hydrodynamic treatments (No Wave and Wave) and 2 nutrient treatments (LN—low nutrient availability and HN—high nutrient availability). See 'Table 2' for more details.

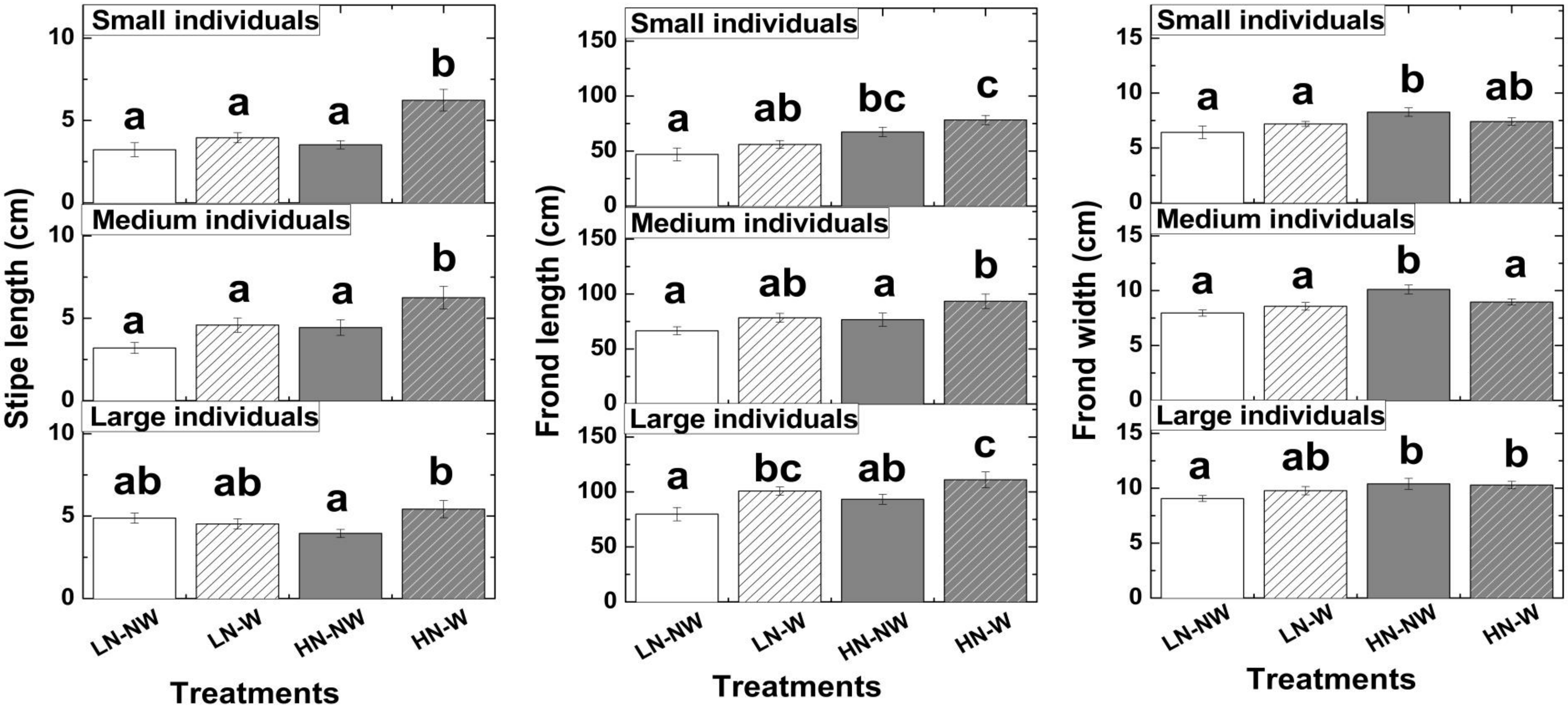

Fig. 4 Morphological traits (stipe length, frond length, and frond width and stipe length) of *S. latissima* under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability). See 'Materials and methods' for more details. Bars represent mean values±1SE (n=8). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan's multiple range test for each size.

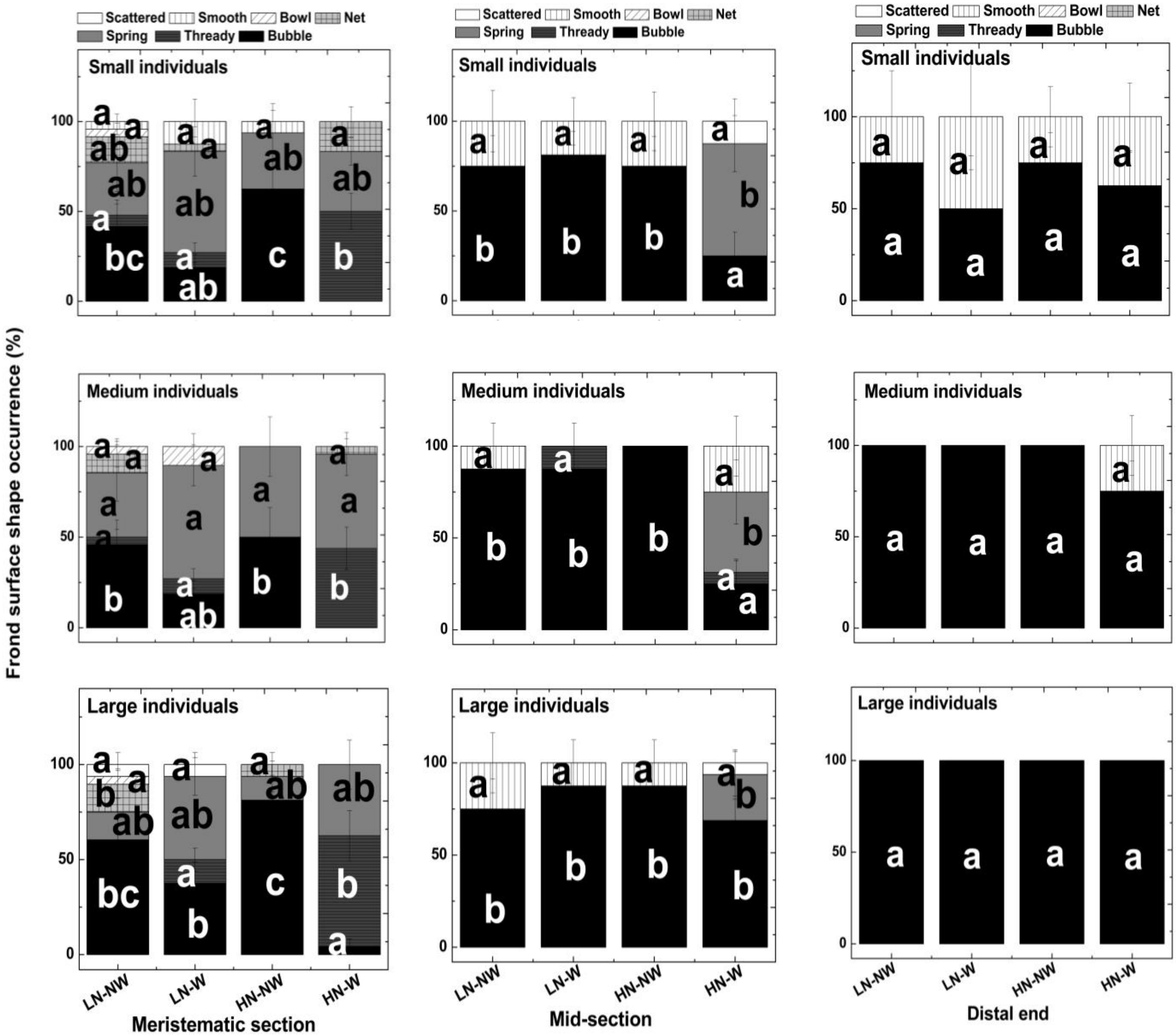

Fig. 5 The occurrence of frond surface shapes in *S. latissima* under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments at the end of the experiment (LN: low nutrient availability and HN: high nutrient availability). See 'Materials and methods' for more details. Bars represent mean values±1SE (n=4,6,7,8). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan's multiple range test for each size and each frond section.

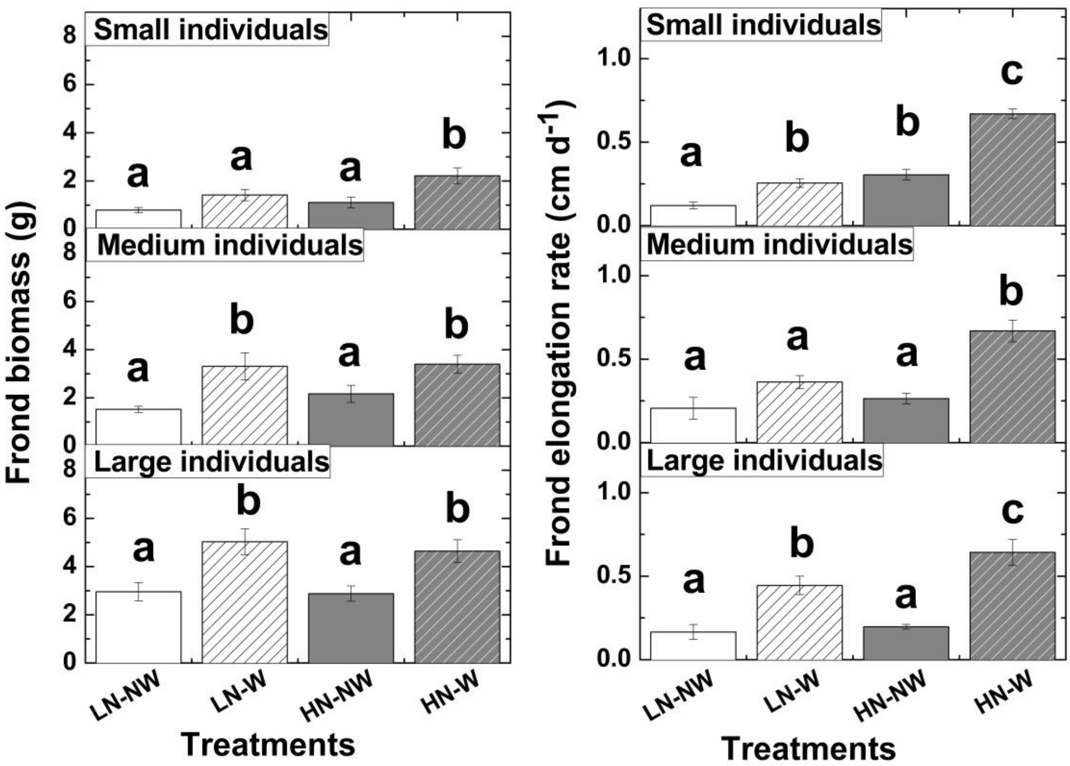

Fig. 6 Growth (frond biomass and elongation rate) of *S. latissima* under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability). See 'Materials and methods' for more details. Bars represent mean values±1SE (n=8). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan's multiple range test for each size.

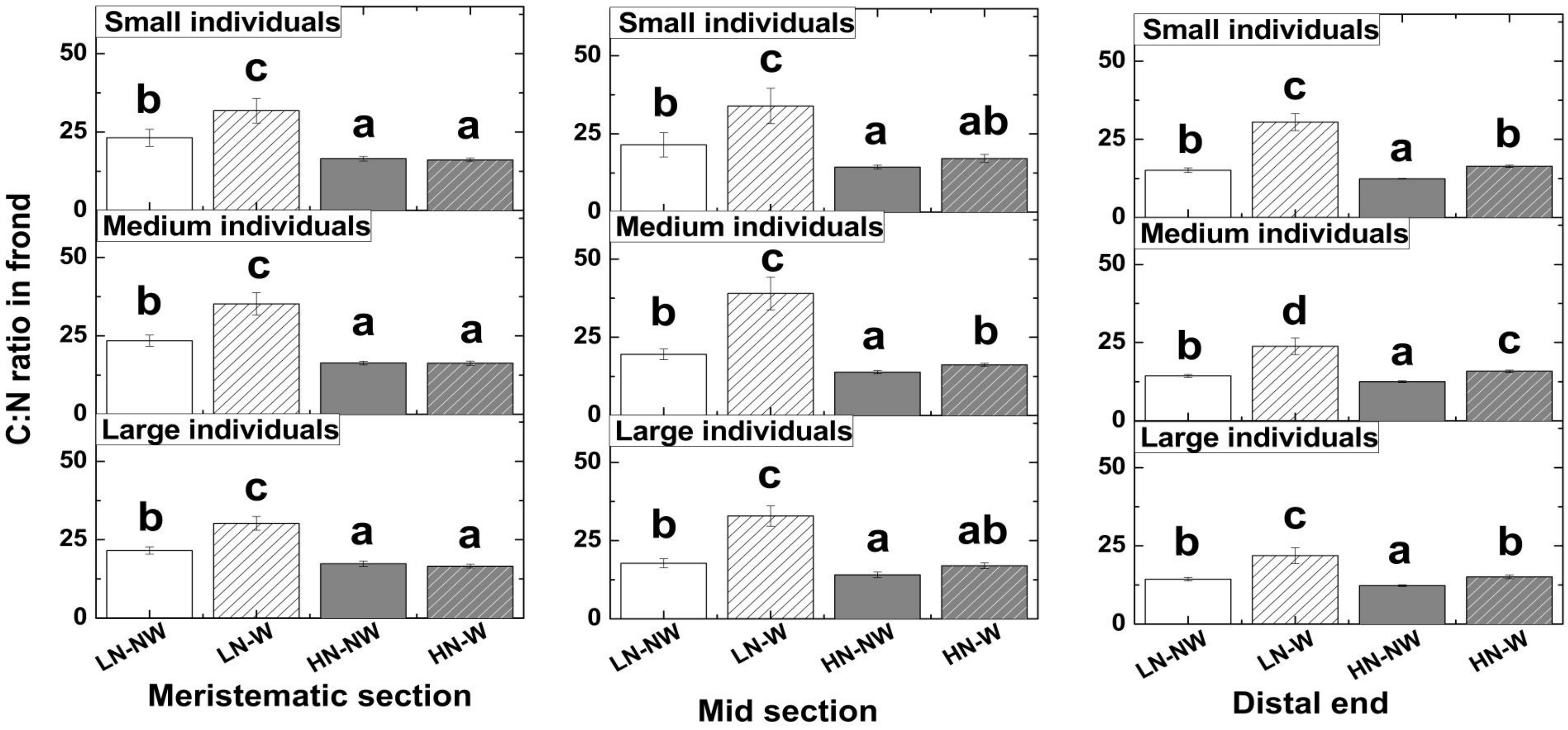

Fig. 7 The C:N ratio in fronds of *S. latissima* under 2 hydrodynamic treatments (NW: no wave and W: wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability) (See 'Materials and methods' for more details). Bars represent mean values±1SE (n=4). Significant differences are indicated by different letters as obtained from one-way ANOVA by combined the 4 treatments together and Duncan's multiple range test for each size and each frond section.

Table 1 The range of nutrient concentrations (mean±1S.E.) under low nutrient conditions (LN), i.e. the filtered seawater without added nutrients during the whole experiment, and high nutrient conditions (HN), with nutrients added to the filtered seawater twice a week, indicated below with values before and after adding nutrients.

	NO_3 (µmol L^{-1})	PO_4 (µmol L^{-1})	*n*
LN			
At the beginning of the experiment	27.92	1.15	1
At the end of the experiment	0.03 ± 0.00	0.40 ± 0.15	4
HN			
Before addition	13.69 ± 1.83	0.11 ± 0.01	68
After addition	34.45 ± 2.28	1.41 ± 0.03	68

Table 2 Categories of frond surface shape in the central frond of *S. latissima* found in this experiment. Shape occurrence (%) was calculated by dividing the frequency of each shape by the total numbers of all types of frond surface shapes in each section.

Shapes	Description	Pictures
Bubble	Bubbles symmetrically and uniformly distributed along both sides of the central frond of the thallus. Some papers have reported this as a common shape type, namely “bullation” or “corrugation”.	
Thready	Bubble connected with each other along the center of frond	
Spring	Bubble connected with each other across the center of frond	
Net	Instead of bubbles, there are lines crossing each other, like in a net.	
Bowl	Part of the frond forms raised or depressed areas, similar to a bowl.	

Smooth	The frond is smooth.	
Scattered	Bubbles uniformly distributed along the frond including the central part.	

Table 3 Effect of wave exposure and nutrient availability on growth and morphological traits of *S. latissima*, tested with two-way ANCOVAs, using size class (SC) as a covariate. Data in **bold** represent statistically significant differences ($P < 0.05$).

Parameter	Size class (*SC*)	Wave exposure (*W*)	Nutrient availability (*N*)	*W*×*N*
Growth				
[a] Frond biomass	**120.53 *** **	**54.20 *** **	3.61 ns	0.10 ns
Frond elongation rate	0.44 ns	**121.78 *** **	**52.29 *** **	**15.05 * **
Morphological traits				
[b] Stipe length	**3.99 * **	**22.81 *** **	**10.03 ** **	3.05 ns
Frond length	**90.66 *** **	**25.38 *** **	**27.00 *** **	0.02 ns
Frond width	**98.16 *** **	0.02 ns	**26.18 *** **	**10.16 ** **
d.f.	1	1	1	1

F-values and their significance are given. Significant differences: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{ns}P > 0.05$, not significant

[a] - ln(x+1)-transformed

[b] - ln(x)-transformed

Table 4 Effects of wave exposure and nutrient availability on the occurrence of categories of frond surface shape and physiological traits of *S. latissima*, tested with two-way ANCOVAs, using size class (SC) and frond section (FS) as covariates. Data in **bold** represent statistically significant differences ($P < 0.05$).

Parameter	Size class (*SC*)	Frond section (*FS*)	Wave exposure (*W*)	Nutrient availability (*N*)	*W*×*N*
The frond surface shape occurrence					
Bubble occurrence	**15.21 *****	**110.47 *****	**34.90 *****	**5.33 ***	**16.66 *****
Thready occurrence	0.18 ns	**46.30 *****	**29.24 *****	**10.14 ****	**12.19 *****
Spring occurrence	2.76 ns	**93.84 *****	**18.08 *****	3.39 ns	1.62 ns
Net occurrence	1.11 ns	**20.94 *****	1.73 ns	0.98 ns	**8.26 ****
Bowl occurrence	0.01 ns	**6.60 ***	0.06 ns	**4.99 ***	0.06 ns
Smooth occurrence	**11.27*****	**6.88 ****	0.02 ns	0.05 ns	0.26 ns
Scattered occurrence	0.19 ns	0.79 ns	1.17 ns	0.13 ns	1.21 ns
Physiological traits					
[a] C:N ratio in frond	0.94 ns	**170.49 *****	**99.75 *****	**204.95 *****	**3.95 ***

d.f.	1	1	1	1	1

F-values and their significance are given. Significant differences: ***P < 0.001; **P < 0.01; *P < 0.05; ns P > 0.05

[a]- reciprocal-transformed

Supplementary data

Tables

Table S1. Effects of wave exposure and nutrient availability on growth performance and general morphology of *S. latissima*, tested with two-way ANOVA for each size class. Data (*F*-values) in **bold** represent statistically significant difference ($P < 0.05$).

Factor	Parameters	Individual Size			*d.f.*
		Small	Medium	Large	
Wave exposure (W)	Frond biomass	**14.52 *****	[A] **20.20 *****	**17.77 *****	1
	Frond elongation rate	**90.32 *****	**28.62 *****	**46.40 *****	1
	Stipe length	**15.33 *****	**10.30 ****	2.44 ns	1
	Frond length	**5.08 ***	**7.47 ***	**12.26 ****	1
	Frond width	[B] 0.39 ns	0.65 ns	0.61 ns	1
Nutrient availability (N)	Frond biomass	**6.00 ***	[A] 1.67ns	0.27ns	1
	Frond elongation rate	**117.94 *****	**12.00 ****	**4.66 ***	1
	Stipe length	**8.67 ****	**8.46 ****	0.00 ns	1
	Frond length	**20.93 *****	**5.77 ***	**4.58 ***	1
	Frond width	[B] **7.45 ***	**14.48 *****	**5.79 ***	1
W×N	Frond biomass	1.15ns	[A] 0.64ns	0.12ns	1
	Frond elongation rate	**16.31 *****	**5.57 ***	2.46 ns	1
	Stipe length	**5.10 ***	0.18 ns	**6.42 ***	1
	Frond length	0.15 ns	0.22 ns	0.09 ns	1
	Frond width	[B] 3.16 ns	**6.99 ***	1.15 ns	1

F-values and their significance are given. Significant differences: $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; $^{ns}P > 0.05$, not significant.

[A]- ln(x+1)-transformed

[B] - square-transformed

Table S2. Effects of wave exposure and nutrient availability on frond surface shape occurrence and physiological traits of *S. latissima*, tested with two-way ANOVA within each frond section and size class. Data in **bold** represent statistically significant differences (*P* < 0.05).

Factor	Parameters		Meristematic section			Mid-section			Distal end			d.f.
			Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	
Wave exposure (W)	The frond surface shape occurrence	Bubble occurrence	**14.09 *****	**11.70 ****	**20.62 *****	2.12 ns	**11.46 ****	0.05 ns	0.73 ns	2.17 ns	.	1
		Thready occurrence	**16.14 *****	**12.39 ****	**23.25 *****	.	1.80 ns	.	.	.	.	1
		Spring occurrence	1.11 ns	1.09 ns	**7.61 ****	**13.64 ****	**6.24 ***	3.50 ns	.	.	.	1
		Net occurrence	0.28 ns	0.59 ns	**4.67** *	.	.	.	.	.	.	1
		Bowl occurrence	1.00 ns	0.59 ns	1.00 ns	.	.	.	.	.	.	1
		Smooth occurrence	0.02 ns	.	.	1.40 ns	0.37 ns	1.08 ns	0.73 ns	2.17 ns	.	1
		Scattered occurrence	.	.	0.00 ns	0.86 ns	.	1.00 ns	.	.	.	1
	Physiological traits	C:N ratio in frond	^{2A} **104.27*****	^{BC} **79.81*****	^{BD} **21.81*****	^{BC} **9.85****	^{B} **32.62*****	^{2A} **28.28*****	^{B}2.98ns	^{B} **5.65***	^{B} **4.35***	1
Nutrient availability (N)	The frond surface shape occurrence	Bubble occurrence	0.01 ns	0.42 ns	0.32 ns	3.50 ns	**5.09 ***	0.05 ns	0.08 ns	2.17 ns	.	1
		Thready occurrence	**7.47 ***	**5.27 ***	**9.74 ****	.	0.20 ns	.	.	.	.	1
		Spring occurrence	0.57 ns	0.02 ns	0.18 ns	**13.64 ****	**6.24 ***	3.50 ns	.	.	.	1
		Net occurrence	0.03 ns	0.59 ns	0.75 ns	.	.	.	.	.	.	1
		Bowl occurrence	1.00 ns	3.21 ns	1.00 ns	.	.	.	.	.	.	1
		Smooth occurrence	0.51 ns	.	.	0.51 ns	0.37 ns	1.08 ns	0.08 ns	2.17 ns	.	1
		Scattered occurrence	.	.	2.00 ns	0.86 ns	.	1.00 ns	.	.	.	1
	Physiological traits	C:N ratio in frond	^{2A} **70.82*****	^{BC} **34.38*****	^{BD}**12.39****	^{BC} **21.39*****	^{B} **70.71*****	^{2A} **34.18*****	^{B} **46.73*****	^{B} **78.20*****	^{B} **59.22*****	1
W×N	The frond surface shape occurrence	Bubble occurrence	3.03 ns	1.03 ns	**6.05** *	3.50 ns	**11.46 ****	1.30 ns	0.08 ns	2.17 ns	.	1
		Thready occurrence	**13.66 *****	**8.45 ****	**9.74 ****	.	0.20 ns	.	.	.	.	1
		Spring occurrence	0.82 ns	0.80 ns	0.05 ns	**13.64 ****	**6.24 ***	3.50 ns	.	.	.	1
		Net occurrence	**5.21 ***	3.21 ns	0.75 ns	.	.	.	.	.	.	1
		Bowl occurrence	1.00 ns	0.59 ns	1.00 ns	.	.	.	.	.	.	1
		Smooth occurrence	1.00 ns	.	.	0.51 ns	3.32 ns	0.00 ns	0.08 ns	2.17 ns	.	1

	Scattered occurrence	.	.	0.00 ns	0.86 ns	.	1.00 ns	.	.	.	1
Physiological traits	C:N ratio in frond	^{2A} 14.37**	^{BC} 0.33ns	^{BD} 0.29ns	^{BC} 0.14ns	^{B} 4.97*	^{2A} 5.98*	^{B} 4.25ns	^{B} 6.46*	^{B} 9.94**	1

F-values and their significance are given. Significant differences: $^{***}P < 0.001$; $^{**}P < 0.01$; $P < 0.05$; $^{ns}P > 0.05$, not significant.

^{A} - ln-transformed.

^{B}-reciprocal-transformed.

^{C}-square-transformed.

^{D}-cube-transformed.

^{2} - the transformed times as certain forms (^{A} or ^{B}).

Table S3. Effects of size class on the growth performance and general morphology of *S. latissima*, tested with one-way ANOVA for each treatment (Table S3a), frond surface shape occurrence and physiological traits of *S. latissima*, tested with one-way ANOVA within each treatment and each frond section (Table S3b). Data in **bold** represent statistically significant difference ($P <$ 0.05), indicated by different letters.

Table S3a

Parameters	Size classes	LN-NW	LN-W	HN-NW	HN-W	*d.f.*
Growth performance						
Frond biomass	Small	a	a	a	a	
	Medium	b	b	b	a	
	Large	c	c	b	b	
	F-values	**21.87*** **	**13.54*** **	**7.04** **	**8.48** **	2
Frond elongation rate	Small		a	b		
	Medium		ab	ab		
	Large		b	a		
	F-values	[2A] 0.39ns	**5.60***	**3.91***	0.06ns	2
General morphology						
Stipe length	Small	a				
	Medium	a				
	Large	b				
	F-values	**7.27** **	0.98ns	1.82ns	0.56ns	2
Frond length	Small	a	a	a	a	
	Medium	b	b	a	ab	
	Large	b	c	b	b	
	F-values	**9.19*** **	**36.94*** **	**6.89** **	**7.20** **	2
Frond width	Small	a	a	a	a	

Medium	b	b	b	b		
Large	c	c	b	c		
F-values	[B] **11.22*** **	**16.92*** **	**7.02** **	**19.64*** **	2	

Table S3b

Parameters		LN-NW Meristematic section	Mid-section	Distal end	LN-W Meristematic section	Mid-section	Distal end	HN-NW Meristematic section	Mid-section	Distal end	HN-W Meristematic section	Mid-section	Distal end	*d.f.*
Frond surface shape occurrence														
	Small						a					a		
Bubble occurrence	Medium						b					a		
	Large						b					b		
	F-value	0.51ns	0.23ns	2.11ns	1.50ns	0.08ns	**6.80** **	1.08ns	1.11ns	2.33ns	1.00ns	**3.61***	1.82ns	2
	Small													
Thready occurrence	Medium													
	Large													
	F-value	0.54ns			0.18ns	1.00ns					0.39ns	1.00ns		2
	Small													
Spring occurrence	Medium													
	Large													
	F-value	0.62ns			0.66ns			1.77ns			0.79ns	1.44ns		2
	Small													
Net occurrence	Medium													
	Large													

	F-value	0.11ns			1.00ns			1.00ns			2.60ns			2
Bowl occurrence	Small													
	Medium													
	Large													
	F-value	0.00ns			2.22ns									
Smooth occurrence	Small						b							
	Medium						a							
	Large						a							
	F-value	1.00ns	0.23ns	2.11ns	1.00ns	0.83ns	**6.80****	1.00ns	1.11ns	2.33ns		2.33ns	1.82ns	2
Scattered occurrence	Small													
	Medium													
	Large													
	F-value	1.00ns			1.00ns							0.60ns		2
Physiological traits														
C:N ratio	Small													
	Medium													
	Large													
	F-value	0.27ns	[C] 0.34ns	0.40ns	0.63ns	0.46ns	2.39ns	0.56ns	0.15ns	0.26ns	0.11ns	0.30ns	1.85ns	2

LN: low nutrient availability; HN: high nutrient availability; NW: no wave; W: wave.

The non-significant differences between size classes were not marked “a” or other same letter (i.e., left blank), to make the table more simple and clearer to read.

[A]- square root-transformed.

[B]-square-transformed.

[C]- reciprocal-transformed.

[2] - the transformed times as certain forms ([A, B] or [C]).

Table S4. Effects of frond section on frond shape occurrence and elemental composition of *S. latissima*, tested with one-way ANOVA within each treatment and each size class. Data in **bold** represent statistically significant differences ($P < 0.05$), indicated by different letters.

Parameters		LN-NW			LN-W			HN-NW			HN-W			*d.f.*
		Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	
Frond surface shape occurrence														
Bubble occurrence	Meristematic section		a		a	a	a		a		a	a	a	
	Mid-section		b		b	b	b		b		ab	a	b	
	Distal end		b		ab	b	b		b		b	b	c	
	F-value	1.30ns	**6.45****	2.76ns	**5.85***	**27.32*****	**11.61*****	0.20ns	**9.33****	0.83ns	**5.78****	**9.80*****	**37.65*****	**2**
Thready occurrence	Meristematic section						b				b	b	b	
	Mid-section						a				a	a	a	
	Distal end						a				a	a	a	
	F-value	0.60ns	0.93ns		1.70ns	0.65ns	4.20*				**25.20*****	**9.47****	**19.06*****	**2**
Spring occurrence	Meristematic section		b	b	b	b	b	b	b		b	b		
	Mid-section		a	a	a	a	a	a	a		b	b		
	Distal end		a	a	a	a	a	a	a		a	a		
	F-value	1.98ns	**4.82***	3.94*	**12.27*****	**30.88*****	**19.42*****	**3.72***	**9.33****	2.33ns	**9.62****	**5.22***	3.17ns	2
Net occurrence	Meristematic section			b							b			
	Mid-section			a							a			
	Distal end			a							a			
	F-value	2.35ns	2.06ns	**3.94***	0.73ns					1.00ns	**4.00***	1.00ns		2
Bowl occurrence	Meristematic section													
	Mid-section													
	Distal end													

	F-value	0.60ns	0.93ns	1.00ns		2.22ns								2
Smooth occurrence	Meristematic section										a			
	Mid-section										a			
	Distal end										b			
	F-value	0.84ns	0.93ns	2.33ns	1.18ns		1.00ns	0.61ns		1.00ns	4.20*	1.17ns		2
Scattered occurrence	Meristematic section													
	Mid-section													
	Distal end													
	F-value			2.33ns			1.00ns				1.00ns		1.00ns	2
Physiological traits														
C:N ratio	Meristematic section		b	c		ab	b	c	b	b				
	Mid-section		b	b		b	b	b	a	a				
	Distal end		a	a		a	a	a	a	a				
	F-value	1.37ns	11.45***	^{A} 10.51***	0.50ns	^{B} 3.88*	4.49*	^{C} 19.02***	14.49***	13.26***	0.34ns	0.20ns	1.87ns	2

LN: low nutrient availability; HN: high nutrient availability; NW: no wave; W: wave.

The non-significant differences between frond section were not marked “a” or other same letter (i.e., left blank), to make the table more simple and clearer to read.

^{A}- ln-transformed.

^{B}-cube-transformed.

^{C}- square-reciprocal-transformed.

Figures

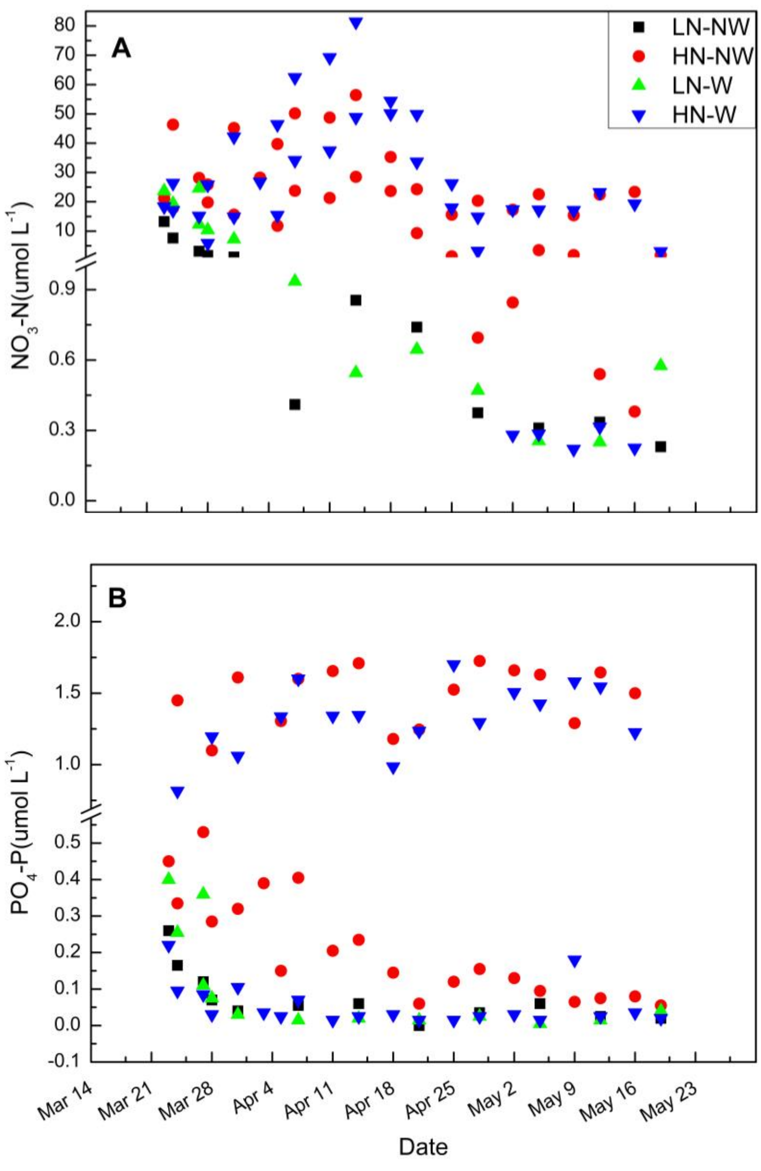

Fig. S1. The concentrations (μmol L^{-1}) of nitrate (A) and phosphate (B) in the seawater under 2 hydrodynamic treatments (W: wave and NW: no wave) and 2 nutrient treatments (LN: low nutrient availability and HN: high nutrient availability) during the experimental period.